Sample Size Selection for the Analysis of Pesticides in Tubers

The determination of representative sample sizes has long been a problem for the food chemist, particularly where trace constituents are concerned. In this study variations in size and surface area, which are the two significant factors affecting the deviation in the concentration of dieldrin within tubers, are quantitatively measured. These measurements are used to demonstrate a method for predetermining sample sizes. The sample sizes developed in this work are applicable particularly to Russet potatoes, though a similar approach can be used for any tubers with pesticides randomly distributed primarily in the skin.

There are many factors affecting the error of an analysis of pesticide residues in tubers: variations in application, sampling methods, recovery, and final identification and quantification. It is important to isolate and measure these errors, not only so that meaningful tolerance levels can be set for food crops, but also so that studies of residue persistence, transport, and metabolic degradation can be optimized.

Sampling of crops for pesticide residues has not been generally subjected to sophisticated, rigorous statistical methods which permit advance determination of the appropriate sample size to yield a preselected precision and confidence limit. General procedures for the sampling of root crops have been developed by Lykken (1963) and such institutions as the FMC Corporation ("Crop Residue Sampling Manual", 1971), but little attempt to indicate the subsequent confidence of the results has been made. Work has been done on the effect of residues of pesticides in the soil upon the final concentration contained in the tubers (Lee, 1968; Sand et al., 1972; Maini et al., 1972). Different methods of application as well as the location of the concentration in the vegetables have been treated. The emphasis here has again been on the total levels of these pesticides, and not on the confidence of the results. Hamilton and Ruthven (1967) discovered that they did not get an improvement in the confidence of their results by taking small portions of many potatoes rather than a corresponding volume of whole potatoes. Taylor and Burrows (1971) have given a discussion of sampling errors in measurements involving the persistence of dieldrin in field soils. Frehse (1975) has given some estimates of the errors to be expected in the final results due to variability in application, sampling, and subsequent analysis steps. However, there is a lack of information allowing the analyst to determine how many samples he should take prior to the analysis itself.

Many researchers determine the appropriate sample size on the basis of availability of crop, labor involved, shipping weight, and other convenience factors, and rely on three or four replicates to provide information about the precision and confidence limits of results. Statistical methods are available which permit prediction of the sample size necessary to achieve the desired precision and confidence limits and which only require that the units of a crop comprising a sample (single apples, cherries, potatoes, etc.) be analyzed as individual units in advance of selection of the sample size. In this paper such a process is shown for root vegetables, and the relative contributions of size and shape or texture variations are isolated.

THEORY

The final concentration of pesticide in a root vegetable depends upon a variety of factors which have been outlined by Edwards (1975). Soil transport rates, moisture content of the soil, and many other parameters play a part and will affect the variance in the concentration measured as well

Chart I. Equations and Definitions[a]

$$C = \frac{4\pi R^2 g / AD}{(4\pi R^3)/3} = km^{-1/3} \tag{1}$$

$$\log C = -\frac{1}{3}\log m + \log k \tag{2}$$

$$\sigma_{\log C} = \frac{1}{3}\sigma_{\log m} \tag{3}$$

$$\sigma_{\log C} = \left(\left(\frac{1}{3}\sigma_{\log m}\right)^2 + (\sigma_d)^2\right)^{1/2} \tag{4}$$

$$n = \frac{t\sigma_{\log C}^2}{\log p} \tag{5}$$

[a] C = concentration of pesticide in the tuber (ppm); R = radius of a spherical tuber (cm); A = area of spherical tuber (cm^2); D = density of tuber (g/cm^3); g = mass of pesticide (g); k = proportionality constant incorporating such factors as the density (g$^{1/3}$); m = mass of the tuber (g); $(\sigma_{\log C})^2$ = variance of the logs of the concentrations; $(\sigma_{\log m})^2$ = variance of the logs of the masses of the tubers; $(\sigma_d)^2$ = contribution to the variance in the log concentration due to surface texture, etc.; n = number of samples (tubers) needed for the analysis (unitless); t = Student's t statistic (unitless); p = the relative precision factor (unitless).

as its mean. Here it is assumed that the soil and pesticide application for any given field will be relatively homogeneous. In this case, the variance in the pesticide concentration within the tubers should depend only upon such factors as variable growth rate and size, surface texture, and translocation. For pesticides which are largely adsorbed upon the skin (Lee, 1968) and which are spherical in shape, the concentration in the tuber depends upon the inverse cube root of the mass (see eq 1, Chart I).

The contributions to the concentration due to differences in surface texture and deviations from spherical shape are more difficult to predict. Thus, such contributions must be measured empirically to determine their significance. Once the variance due to these factors has been estimated or measured, the final variance in the concentration can be obtained by summing up the individual contributions.

Before relating the variance in the concentration to the variance in the size by propagation of error mathematics, it will be convenient to normalize our variables. Then confidence limits can be more directly and easily obtained from the observed standard deviations. For determinations involving concentrations of trace components of sizes of objects where it is impossible to have zero or negative values, the log-normal distribution applies (Ahrens, 1954). By taking the logs of the concentrations and the logs of the masses of the tubers, we will obtain variables which are more normally distributed, and which will be related

to each other by eq 2 (Chart I). Propagation of the standard deviation gives the relationship in eq 3 (Chart I). Adding on the contribution due to texture and shape deviations provides the final equation (4), where σ_d is the standard deviation in the log of the concentration due to surface texture and shape deviations. This factor should vary from one species and grade of vegetable to another, but should be relatively constant for, e.g., U.S. 1 Russet potatoes. Once we know the standard deviation for the distribution in the masses of the tubers and σ_d, we can predict the standard deviation in the concentration as well. More importantly, the concentration confidence intervals can be estimated for any mean concentration if the number of tubers sampled and the distribution of masses and the shape and texture factor are known. Conversely, the number of samples needed for any desired confidence interval can be estimated. This will be of great potential usefulness to the analyst.

EXPERIMENTAL PROCEDURE

Individual Russet potatoes from one field known to have a dieldrin residue were randomly selected and washed but not scrubbed under tap water. Each was chopped and homogenized with a food blender and 100 g (or as much sample as could be collected for the smaller potatoes) was weighed for the analyses. One-hundred milliliters each of acetone and hexane were added to each sample and the resultant mixture was stirred with a high-speed blender for 5 min and the mash was then left to separate for 1 h.

The extract was filtered from the mash and the acetone was removed with three 100-ml washes of 3% NaCl in a separatory funnel. The hexane layer was then evaporated to a final volume of 10 ml. The resulting concentrate was cleaned using a 10-g 1:1 Magnesia–Celite column with a Na_2SO_4 plug and eluted with 200 ml of hexane. The final volume was brought up to 250 ml with hexane and the samples were analyzed by GC for dieldrin with an OV 210:17–8:2% column with an electron capture detector. An 80–90% recovery of pesticide was obtained with this procedure. Identity of the pesticide was confirmed with retention times on three columns, p values, internal standards, and mass spectroscopy.

A second sample of potatoes, all U.S. 1 Russets with negligible dieldrin residues, was dipped in a 10-ppm dieldrin solution in hexane so that a uniform coating of dieldrin was obtained on the surface and the final concentrations of pesticide in the tubers were comparable to those obtained from the field samples. These were also analyzed according to the procedure outlined above.

RESULTS

The potatoes were from one field, and met the assumptions given, except for deviations from a spherical shape. No attempt was made to eliminate the odd shapes from the analysis. It is important to leave these odd shapes in the analysis since they affect the variance by reducing the dependence of the concentration on the mass.

Twenty-three of these potatoes were individually analyzed and the logs of the concentrations vs. the logs of the masses have been plotted in Figure 1. The log-normal means for the 23 samples' masses and concentrations are 2.32 and −7.63, respectively, corresponding to a mean mass of 209 g and a mean concentration of dieldrin of 0.023 ppm. A linear regression through the points gives a slope of −0.328 vs. the predicted −0.333, showing excellent (and somewhat fortuitous) agreement. Therefore, the assumption that the concentration varies with the inverse cube root of the mass is found to be valid. (If the apparently extraneous point at the upper left is discarded, the slope is not significantly different.)
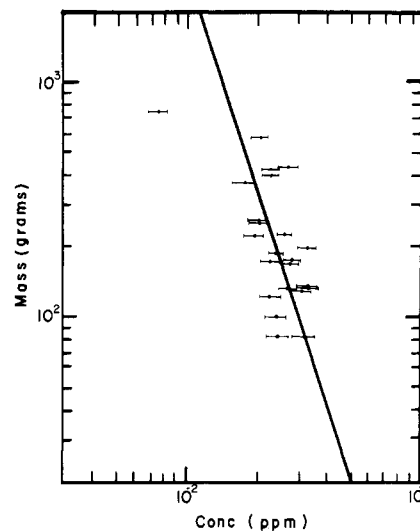

Figure 1. Dependence of the concentration of the pesticide on the mass of the tuber for 23 potatoes. Error brackets correspond to the final analysis error.

Twenty potatoes were dipped in a pesticide solution to provide an estimate of the variance due to surface texture and shape inhomogeneities. The log-normal means of the masses and concentrations are 2.29 and −7.66, respectively, corresponding to a mean mass of 195 g and a mean concentration of dieldrin of 0.022 ppm. The variance about the line of best fit (9.6×10^{-3}) is due only to variations in the dipping procedure (assumed to be negligible), the analysis step (found to be negligible), and differences in the surface texture and shape. Thus, substitution of this variance and the variance due to differences in the size (mass) of the potatoes into eq 4 (Chart I) should predict the total variance in the concentration of the pesticide in the potatoes, if our assumptions are valid. The measured population variance in the log of the concentration for the field samples was 1.8×10^{-2}, corresponding to a range of concentrations from 0.017 to 0.032 ppm for one standard deviation. The predicted population variance of 1.2×10^{-2} corresponds to a range of concentrations from 0.018 to 0.030 ppm. As can be seen, there is no significant difference between these variances.

The importance of these results is that by two simple measurements, a determination of the size (mass) distribution and a determination of shape and texture variability, the error of sampling and analyzing any crop which has pesticide distributed primarily upon the surface can be estimated. This work implies that soil inhomogeneities, application variability, translocation, and other factors contribute less to the variance than the variations in mass and texture. These two variables need be measured only once for a given type and grade of crop. If comparisons are being made between one locality and another, a third factor covering climate, soil, and other variables will probably be significant, but most fields will have only one soil type, with fairly uniform pesticide concentrations. If portions of a field have different irrigation, soil types, or other differences, the contributions to the total variance from these sources may not be negligible.

Once we have established the major sources of the variance we can calculate how many samples are needed for a given level of precision and variance using the log-normal distribution, as shown in eq 5 (Chart I). The value for t will determine the confidence level, and the value for p will determine within what factor we will bracket the mean. For instance, if we are willing to say that our results lie within the range 50 to 200 ppm, with a mean of 100

Table I. Number of Potatoes Needed for a Given Confidence and Precision[a]

Precision factor	Confidence level, %			
	68	80	90	95
1.05	27	44	72	100
1.10	7	13	21	29
1.20	2	5	7	10
1.50	1	2	3	4
2.00	1	2	3	3

[a] Number of samples has been rounded off to the nearest whole potato, and sample numbers less than one are rounded up to one.

ppm, we will have an error or range within a relative precision factor of 2. Or, for a precision factor of 1.1, we would expect the results to lie between 91 and 110 ppm for a mean of 100 ppm. The number of samples predicted in this manner clearly holds for any concentration of pesticide where the deviation in tuber size, shape, and texture limits the precision of analysis, and the pesticide is distributed in or on the skin.

Table I gives sample size vs. the relative precision factor for various confidence levels. In order to use the table, one has only to decide upon a precision factor and confidence level which are acceptable, and the minimum number of samples can be obtained directly. Often the economics of the situation will be such that a compromise will be necessary, and the highest confidence level will be selected which does not require too many samples. Despite such limitations, the efficiency of such a table is far superior to a blanket "15 potatoes" ("Crop Residue Sampling Manual", 1971) or "50 lbs. of potatoes" (Lykken, 1963) for recommending sample sizes.

Although only one population of potatoes was examined here, the log-normal distribution is relatively insensitive to the origin of the tubers, and the table for determining sample sizes should apply to other fields, for different concentrations of a variety of pesticides. Tubers with shapes and textures that deviate greatly from those used here can still be analyzed with this approach, although empirical measurements should be made to estimate σ_d and σ_m. For maximum efficiency in sampling it is always best to calculate one's own sampling tables from eq 4, if the relevant data are available.

This theory is in conformance with values reported in the literature or obtained by other observers. Preliminary measurements and values extracted from the literature for sweet potatoes and sugar beets indicate that at least these tubers also follow the assumptions made here, and useful approximations for the sample size needed for an analysis can be obtained from Table I. More verification and measurements are needed to extend the work to other root crops, but the approach taken here should prove to be quite useful.

Finally, it is important to realize that the concentrations of trace components such as pesticides are distributed log normally. If only the mean is reported (a deplorable practice), this makes little difference. If confidence intervals or standard deviations are to be reported as well,

it is necessary to give the range as calculated using the log-normal interval.

CONCLUSIONS

For too long pesticide residue analysts have had to be satisfied with the results of an analysis, having no idea how many samples were needed until a survey sampling was run. Large numbers of replicates can be unnecessarily expensive. Table I should alleviate this problem by providing estimates of the number of samples needed for a desired confidence level and precision factor.

The relationship between size and concentration also points out how important it is to select samples randomly with respect to size. If only a certain size range, such as bakers, is to be analyzed, it should be realized that the results are not representative of the field as a whole. (Corrections can be made, if the distribution of sizes is known, from the inverse cube root relationship.)

Finally, it is important to realize that the approach taken here is applicable even if soil inhomogeneities or other factors prove to be significant, as their variances can be summed up as shown in eq 4. Sampling problems can thus be handled in a manner similar to that demonstrated here, increasing the efficiency and usefulness of the analysis.

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